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In vitro secretion of human chorionic gonadotrophin by bladder tumour cells

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Summary Human chorionic gonadotrophin (hCG) and alphafetoprotein (AFP) were measured in culture media from a panel of 29 cell lines including 9 bladder carcinomas, 5 'normal' bladder epithelia, 10 germ cell tumours, and 5 miscellaneous tumours and 'normal' cell lines. In 7 of the 9 bladder carcinomas and 4 of the 5 'normal' bladder epithelia, the media contained hCG at levels ranging from between 34 and 3,600 $IU l^{-1}$. All other cell lines, including the 10 germ cell tumour lines gave negative results for hCG. These findings indicate that *in vitro* secretion of hCG is a common feature of normal and neoplastic bladder transitional epithelia, and support the hypothesis that parts of the genito-urinary epithelium have a potential for hCG production.

The high incidence of elevated levels of human chorionic gonadotrophin (hCG) and alphafetoprotein (AFP) in association with testicular germ cell tumours is well-recognised *in vivo* (Seppala *et al.*, 1986), but this phenomenon is relatively uncommon during culture of these tumours (Andrews *et al.*, 1980). Ectopic production of hCG is also a well-recognised phenomenon in some non-gonadal epithelial tumours (Braunstein *et al.*, 1973; McManus *et al.*, 1976; Bellet *et al.*, 1980). This is particularly common in gastric (24%), hepatic (17%) and pancreatic (50%) cancers (reviewed by Baylin & Mendelsohn, 1980; Heyderman *et al.*, 1985; Seppala, 1986). The incidence may be even higher with malignant non-gonadal cells *in vitro*; in one such study (Rosen *et al.*, 1980) 19 of 32 tumour cell lines secreted hCG (including one bladder carcinoma line). A few isolated cases of *in vivo* elevated hCG levels in serum and urine in association with bladder cancer have been reported (Civantos & Rywlin, 1972; Kawamura *et al.*, 1978; Rodenburg *et al.*, 1985; Norman *et al.*, 1985). Very recently measurement of hCG has been used as a marker for monitoring metastatic bladder disease; 28 of 92 patients demonstrated measurable levels in blood (Dexeus *et al.*, 1986). Immunohistochemical studies suggest that ectopic production of hCG is relatively unusual in bladder tumours with reports varying from 5 of 13 to 12 of 104 cases (Rodenburg *et al.*, 1985; Shah *et al.*, 1986). In the present study we have examined hCG and AFP secretion by normal and neoplastic bladder and testicular germ cell tumours *in vitro*.

Materials and methods

A total of 29 cell lines were examined in this study (Table I). They included: 14 lines of urothelial origin (9 neoplastic (see Table I), 5 'normal'); 10 germ cell tumours (gonadal) and 5 miscellaneous tumours and 'normal' controls. The American Type Culture Collection (ATCC) and other lines held at the London Hospital were grown in a medium consisting of Leibowitz L-15 RPMI 1640 (50/50, v/v) containing 20% heat inactivated foetal calf serum (FCS), transferrin (4 $\mu g ml^{-1}$), hydrocortisone (4 $ng ml^{-1}$), insulin (4 $\mu g ml^{-1}$), and penicillin-streptomycin (1,000 $U ml^{-1}$) (Gibco Ltd., Paisley, Scotland). Cell lines from the Institute of Urology were grown RPMI 1640 plus 5% FCS (Gibco). Incubation was carried out at 37°C in a mixture of 95% air and 5% CO_2. The cell lines were grown to confluence in culture flasks (75 cm adherence area; Falcon Labware).

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Table I Cell lines used in study.

Cell lines	*Origin*	*Source*	*Beta-hCG* ($IU l^{-1}$)	*AFP* ($U ml^{-1}$)
(A) *Urothelial Lines*				
Neoplastic				
T24	Tcc bladder	ATCC	<25	<10
J82	Tcc bladder	ATCC	56	<10
RT4	Tcc bladder	ATCC	34	<10
TccSUP	Tcc bladder	ATCC	34	<10
ScaBER	Scc bladder	ATCC	2400	<10
5637	Tcc bladder	ATCC	220	<10
RT112	Tcc bladder	ATCC	220	<10
HT1376	Tcc bladder	Inst Ur	<25	<10
TccDeS	Tcc bladder	LH(1)	3600	<10
'Normal'				
HS0767	TcE bladder	Inst Ur	<25	15
HU609	TcE bladder	Inst Ur	1150	<10
NB/AJ	TcE bladder	LH(2)	130	<10
NB/UI	TcE bladder	LH(2)	60	<10
NB/U2	TcE bladder	LH(2)	70	<10
(B) *Germ Cell Tumours and Controls*				
GCTs				
GCT27	GCT	Inst Ur	<25	<10
1618K	GCT	Inst Ur	<25	<10
HL	GCT	Inst Ur	<25	<10
833K	GCT	Inst Ur	<25	<10
GH	GCT	Inst Ur	<25	20
TERA II	GCT	ATCC	<25	<10
TERA I	GCT	ATCC	<25	16
SuSa	GCT	Inst Ur	<25	18
WG007	GCT	LH(1)	<25	<10
PJ077	GCT	LH(1)	<25	<10
Controls				
'Normal'				
UV/K14	Skin (keratinocyte)	LH(2)	<25	<10
Malme 3	Skin (fibroblast)	ATCC	<25	<10
Neoplastic				
WTu013	Wilms tumour	LH(1)	<25	13
BrCaPE	Breast carcinoma	LH(1)	<25	<10
MJ003	Breast carcinoma	LH(1)	<25	<10

Tcc = Transitional cell carcinoma. Scc = Squamous cell carcinoma. TcE = Transitional cell epithelia. GCT = Germ cell tumour. ATCC = American Type Culture Collection. Inst Ur = Institute of Urology. LH(1) = London Hospital originated by R. Iles. LH(2) = London Hospital originated by I. Leigh & P. Purkis, Department of Dermatology.

NB. Cell lines NB/AJ, NB/UI, NB/U2 used at Passage 3 of origin and cell line TccDeS at pre-passage primary cultures.

At this stage the medium (10 ml) was exchanged and the culture continued for a further 96 h. The medium was then harvested and, after removal of debris by centrifugation, was stored at −30°C until assayed. AFP was measured by radioimmunoassay (RIA) (Chard, 1978) and hCG by RIA directed to the beta subunit of this molecule (Norman *et al.*, 1985). The minimal detection limit of the latter assay is 25 IU l^{-1}.

Results

Media from 7 of the 9 bladder cancer cell lines contained detectable levels of beta-hCG (Table IA; Figure 1). In 2 of these (TccSUP and RT4) the concentrations were only marginally above controls but in the remainder the levels were substantially raised. Four of the 5 'normal' bladder epithelial cell lines also showed levels of beta-hCG that were markedly elevated. The media from the germ cell tumours and other cell lines examined all gave negative results (Figure 1). Media containing hCG levels greater than 50 IU l^{-1} were also assessed using a semi-quantitative two-site immunometric assay (Hybritech ICON). All gave negative results. Within the bladder cell lines there appeared to be a general association of epithelial morphology and high levels of beta-hCG (Table II). The occasional positive level of AFP (Table I) was only marginally different from the 'noise' level of the assay.

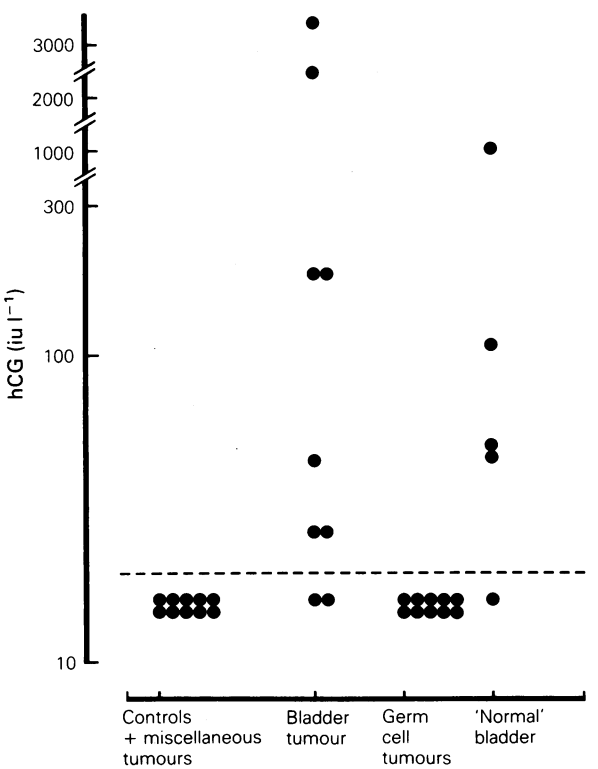

Figure 1 Levels of hCG in the media from 9 lines of cultured bladder tumours (see **Table I**), 10 germ cell tumours, 5 normal bladder cell lines, and 10 controls (5 cell lines and 5 media controls).

Discussion

There is good evidence that hCG may be produced by normal tissues (Yoshimoto *et al.*, 1977, 1979*a*). Furthermore, using assays of exceptional sensitivity and specificity (i.e. as low as 0.01 ng ml^{-1}), hCG can be detected in the urine of some normal non-pregnant subjects, post-menopausal females and women using oral contraceptive agents (Chen *et al.*, 1976; Armstrong *et al.*, 1984; Huang *et al.*, 1984). Significant amounts of hCG (and other oncoplacental proteins) are also found in seminal plasma (Salem *et al.*, 1984). These studies have led to the proposal that most tissues are capable of synthesising hCG, and especially some of the epithelia bordering the genito-urinary tract.

The present study confirms previous observations on the secretion of hCG by non-gonadal malignant cells *in vitro* (Rosen *et al.*, 1980) and highlights the lack of *in vitro* secretion by the testicular germ cell tumour lines in long term culture (Andrews *et al.*, 1980; Cotte *et al.*, 1981). Given the basic ability of all cells to synthesise hCG and the derepression of this ability in many tumours (Yoshimoto, 1979*b*) it is not surprising that we have observed hCG secretion by bladder tumour cells *in vitro*. However, the secretion by 'normal' urothelial cell lines implies an innate capacity for hCG secretion. This is further supported by the apparent high frequency of this phenomenon (7 of 9 bladder carcinoma cell lines and 4 of 5 'normal' bladder epithelia), especially when other cell lines, including the testicular germ cell tumours, were negative in this respect. If hCG secretion is a characteristic feature of bladder epithelial cells in culture, it would support the hypothesis that hCG secretion is a characteristic of the normal and neoplastic urothelium. The primary cell line TccDeS (beta-hCG 3,600 IU l^{-1}) provides a link between the *in vivo* and *in vitro* situation. It was isolated from the pleural effusion of a patient with metastatic bladder cancer who had substantial levels of hCG in both blood and pleural fluid (760 and 2,700 IU l^{-1} respectively.

The apparent discrepancy between the secretion of hCG by germ cell tumours *in vitro* and *in vivo* may be a consequence of the very different micro-environment *in vivo* allowing the tumour cells to differentiate into trophoblast (Bronson *et al.*, 1983; Volgelzang *et al.*, 1983; McIlhinney, 1983). At the same time, a marginal increase of AFP does seem to be a feature of germ cell tumours *in vitro* (Table IB; (Andrews *et al.*, 1980).

We have not as yet attempted a full molecular characterisation of the 'hCG' detected in the cell media.

Table II Bladder cancer cell lines – morphology and histology

Cell line	*Cell morphology*	*Grade*	*Stage*	*hCG levels (IU l^{-1})*
SCaBER	Large regular epithelial cells		T3	2400
5637	Small regular epithelial cells	NR	NR	220
RT112	Small regular epithelial cells	I/II	NR	220
RT4	Small irregular epithelial cells	I/II	T1	34
J82	Mixed epithelial and fibroblastic-like cells	III	T3	56
TccSUP	Mixed epithelial and fibroblastic-like cells	IV+	T4	34
T24	Mixed epithelial and fibroblastic-like cells	III	NR	<25
HT1376	Variable sized regular epithelial cells	III	T2/3	<25
TccDeS	Variable sized regular epithelial cells	II/III[a]	T4	3600

NR = not reported. Histology survey: Hepburn & Masters, 1983; Masters *et al.*, 1986. [a]Institute of Pathology, The London Hospital.

Nevertheless, the fact that high levels were determined by an assay which is specific for the beta-chain, while negative results were obtained with a two-site assay specific for intact hCG, strongly suggests that the material consists principally of the beta subunit or fragments thereof. It is interesting that of the five ATCC (1985 catalogue) cell lines listed as hCG secretors, 4 are of placental/foetal origin (3 choriocarcinomas and one line of SV/40 transformed normal placental cells) and one is a cervical epidermoid carcinoma. The latter non-embryonal tumour cell line (CaSk1) is reported to secrete the beta subunit than intact hCG (Pattillo *et al.*, 1977). In a previous study, the hCG-like material found in urine and blood from patients with germ cell tumours appeared to be mainly intact hCG, while material from the patient with a metastatic bladder tumour consisted principally of free beta subunit (Norman *et al.*, 1985). Variability of the relative concentration of intact hormone and free subunits, together with heterogeneous glycosylation of the protein chains, has also been reported in studies on patients with a variety of other tumour types (Weintraub & Rosen, 1973; Rosen & Weintraub, 1974; Kahn *et al.*, 1977; Yoshimoto *et al.*, 1979*b*). The degree of glycosylation affects various biochemical characteristics and also the rate of metabolism *in vivo* (Van Hall *et al.*, 1971; Tsuruhara *et al.*, 1972). Independent production of subunit and intact hormone is not surprising as there is evidence that the genes for the alpha and beta subunits may be on different chromosomes. Thus, the alpha chain has been cloned (Fiddes & Goodman, 1979) and tentatively assigned to chromosome 6 (Naylor *et al.*, 1984), whereas the beta chain, also cloned (Fiddes *et al.*, 1980) has been mapped to chromosome 19 (Julier *et al.*, 1984). Furthermore, the beta hCG 'gene' is itself very heterogeneous consisting of a cluster of at least eight genes arranged in tandem and inverted pairs; one of this cluster is the gene for the beta chain of luteinising hormone (LH) (Boorstein *et al.*, 1982; Talmadge *et al.*, 1983).

The significance of this genetic complexity is unclear at the present time (Talmadge *et al.*, 1984; Whitfield & Kourides, 1985) but may partly explain the very variable results reported here and in the literature.

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